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Isolation of a Purified Skin Test Antigen from *Blastomyces dermatitidis* Yeast-Phase Cell Wall

MICHAEL V. LANCASTER[1]* AND RONALD F. SPROUSE[2]

Department of Veterinary Microbiology and Department of Medical Microbiology, University of Missouri, Columbia, Missouri 65201

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Preparative polyacrylamide gel electrophoresis was used to isolate individual components of an alkaline-soluble–water-soluble fraction of the cell wall of *Blastomyces dermatitidis* yeast phase. One component isolated demonstrated exceptional specificity and reactivity when tested on guinea pigs infected with *B. dermatitidis*. This component displayed no cross-reactivity when tested on animals infected with *Histoplasma capsulatum*. The significance of isolation of a purified, specific antigen is discussed.

The requirement for a reliable immunological tool useful in epidemiological surveillance, serodiagnosis, and diagnosis of blastomycosis long has been obvious. Past attempts to purify antigenic components generally have been limited to analysis of crude mycelial filtrates of *Blastomyces dermatitidis* (4, 6, 7). However, Cox and Larsh (1) recently have succeeded in extracting a partially purified cell wall extract (designated ASWS PM-10) from the yeast phase of *B. dermatitidis* with increased reactivity and specificity in guinea pigs sensitized with merthiolate-killed yeast cells of *B. dermatitidis*. Although this alkaline-soluble–water-soluble (ASWS) extract is only partially purified, its skin test reactivity appears to be consistent from preparation to preparation and consequently warrants additional examination.

Preparative-scale polyacrylamide electrophoresis was judged a suitable separation technique for obtaining purified components from the ASWS extract since the extract had already been characterized by analytical polyacrylamide disc electrophoresis (2). Thus, the objective of this study was to isolate purified components from the ASWS extract of *B. dermatitidis* yeast cell walls by preparative-scale polyacrylamide electrophoresis and to evaluate the skin test reactivity and specificity of those components.

MATERIALS AND METHODS

Antigen fractionation. The ASWS antigen was prepared from *B. dermatitidis* by the procedure described by Cox and Larsh (1). Once the ASWS antigen had been prepared, it was applied directly to a preparative disc electrophoresis system. A 5% polyacrylamide gel containing tris(hydroxymethyl)aminomethane-hydrochloride buffer (pH 8.9, 0.014 M) was photopolymerized in a preparative electrophoresis column (2.5 by 15 cm). Tris(hydroxymethyl)aminomethane-glycine buffer (pH 8.9, 0.015 M) was used for electrode and elution buffers. ASWS antigen (1.2 ml, 10 mg/ml) was layered on the gel surface. The anode of a constant-current power supply was connected to the lower chamber, and the cathode was connected to the upper buffer chamber. Elution buffer (pH 8.9) was pumped across the lower end of the gel at 15 ml/h. Power was then applied to the column (1,000 V at 10 mA; 10 W), and electrophoresis was continued for 16 h. Column effluent was monitored by ultraviolet absorbance at 280 nm and was collected in a fraction collector. Consecutive fractions showing 280-nm absorbance were pooled, dialyzed against distilled water, and lyophilized. The fractions collected were subsequently analyzed by analytical disc electrophoresis according to the method of Davis (3). Gels were stained with amido black 10B in 7% acetic acid and destained in 7% acetic acid. Stained zones were recorded with a densitometer.

Skin testing of pooled fractions. Pooled electrophoretic fractions of the ASWS extract were assayed for skin test reactivity by intradermal inoculation (approximately 5 μg of protein dissolved in 0.1 ml of sterile saline) of guinea pigs infected with either *B. dermatitidis* or *Histoplasma capsulatum* (5). All pooled fractions were tested on *Blastomyces*-infected pigs, *Histoplasma*-infected pigs, and noninfected pigs. Blastomycin KCB-26 (1:100) was applied to *Blastomyces*-infected pigs as positive controls. Induration was recorded at 24 and 48 h after inoculation. Induration readings of 48 h were used for statistical calculations and for interpretation of results.

RESULTS

Figure 1 represents the elution profile of the preparative electrophoresis and separation of

[1] Present address: Department of Dermatology Research, Letterman Army Institute of Research, Presidio of San Francisco, CA 94129.

[2] Present address: Departments of Pathology and Microbiology, School of Medicine, University of Missouri, Columbia, MO 65201.

ASWS antigen. Four peaks absorbing at 280 nm were apparent. All four eluted from the column within 8 h of initiation of electrophoresis. Figure 2 represents densitometer tracing of the analytical disc electrophoresis of the pooled fractions and unfractionated ASWS antigen. The unfractionated ASWS tracing shows at least four peaks, designated f_1, f_2, f_3, and f_4, with R_f values of 0.99, 0.77, 0.53, and 0.11, respectively. Densitometer tracings of the pooled fractions demonstrate identity of those fractions to the four zones in the unfractionated ASWS. In addition, no cross-contamination is apparent in any of the pooled fractions.

Table 1 represents the results of skin testing the pooled fractions. Controls were essentially negative, with only one animal reacting to ASWS and ASWS f_3. Inspection of the data in Table 1 suggests ASWS f_4 to be a superior antigen to the ASWS unfractionated antigen on a weight-to-weight basis. The cross-reactivity of ASWS appears to be present in f_1, f_2, and f_3 but absent in f_4. Data presented in Table 2 further support the superiority of ASWS f_4. Whereas ASWS reacted in 73% (8/11) of the *Blastomyces*-infected animals, it also reacted in 2 of 10 *Histoplasma*-infected animals. ASWS f_4 reacted in 100% (11/11) of the *Blastomyces*-infected group while reacting on none of the *Histoplasma* group. Further, the range of induration size for ASWS f_4 in the *Blastomyces*-infected group was 9 to 11 mm. Statistical analysis indicated no significant difference ($P > 0.05$) in average indurations among the ASWS fractions tested on *Blastomyces*-infected animals. ASWS f_1, f_2, and f_3 showed no significant differences for each antigen between the reactions elicited on *Blastomyces*-infected and *Histoplasma*-infected animals. Both unfractionated ASWS and ASWS f_4

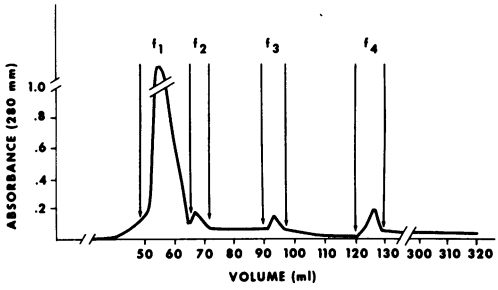

FIG. 1. *Elution pattern of Blastomyces cell wall fraction ASWS PM-10 from preparative polyacrylamide gel electrophoresis.*

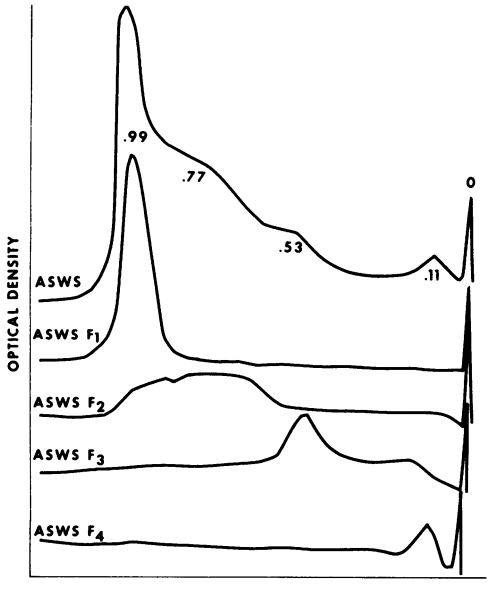

FIG. 2. *Densitometer tracings of Blastomyces cell wall ASWS PM-10 fractions from preparative electrophoresis. Origin is designated by O. R_f values of four components are shown.*

TABLE 1. *Results of skin testing Blastomyces cell wall fractions*

Antigen	Concn (mg of protein/0.1 ml)	Induration[a] (48 h)		
		Control[b]	*Blastomyces* infected[c]	*Histoplasma* infected[d]
Saline	0.0	0.0	0.0	0.0
KCB-26	1:100 dilution	0.0	9.4	NT[e]
HKC-5	1:100 dilution	0.0	NT	12.2
ASWS	5.0	1.2	8.6	2.2
ASWS f_1	5.0	0.0	5.4	2.4
ASWS f_2	5.0	0.0	7.2	5.2
ASWS f_3	5.0	1.2	7.8	7.2
ASWS f_4	5.0	0.0	9.6	0.0

[a] Measurement represents mean induration.
[b] Nine guinea pigs were tested.
[c] Eleven guinea pigs were tested.
[d] Ten guinea pigs were tested.
[e] NT, Not tested.

TABLE 2. *Number and percentage of reactors to Blastomyces cell wall fractions*

Antigen	No. (%) of reactors at 48 h		
	Control	*Blastomyces* infected	*Histoplasma* infected
ASWS	1/9 (11)	8/11 (73)	2/10 (20)
ASWS f_1	0/9 (0)	6/11 (55)	4/10 (40)
ASWS f_2	0/9 (0)	8/11 (73)	6/10 (60)
ASWS f_3	1/9 (11)	9/11 (82)	9/10 (90)
ASWS f_4	0/9 (0)	11/11 (100)	0/10 (0)

elicited reactions that were significantly larger in the *Blastomyces*-infected group than in the *Histoplasma*-infected group.

DISCUSSION

This study has presented evidence that a single component isolated from the cell wall of the yeast phase of *B. dermatitidis* is both reactive and specific when used as a skin test agent to differentiate *Blastomyces* infections from *Histoplasma* infections in guinea pigs. The component was shown superior to unfractionated ASWS with regard to specificity and reactivity and was at least as good as KCB-26.

Comparison of our results with those presented by Cox and Larsh (1) reveal similarity in specificity of the ASWS antigen. In both studies the antigen reacted in about 80% of the *Blastomyces*-infected animals and 10% of the *Histoplasma*-infected animals. However, maximal reaction occurred at 24 h in the Cox and Larsh study and at 48 h in our study. In the earlier study, 100 μg of material was required, whereas our study used only 5 μg of protein for each skin test. Both differences may be attributed to the difference in methods used to sensitize the guinea pigs. Cox and Larsh used guinea pigs that had been sensitized to *Blastomyces* and *Histoplasma* yeast, whereas this study used animals that were infected and represented active blastomycosis and histoplasmosis.

One might expect antigens prepared from whole yeast cells to exhibit a high degree of specificity when tested on animals sensitized with killed homologous yeast cells as compared with animals infected with hyphal-phase cells. Sensitization of the test animals with yeast cells precludes initial exposure to the pathogen's infective particles and subsequent morphological stages as the organism converts in vivo to the yeast phase. Such changes in morphology could, conceivably, be important sources of antigenic stimulation. Further, since infected animals carry the organism for extended periods, greater sensitivity to skin testing would be expected in infected animals. Cox and Larsh produced an antigen that was more specific and consistent than earlier blastomycins. The use of animals sensitized with homologous organisms rather than chronically infected with hyphal-phase particles could lead to higher specificity than shown by blastomycin. In addition, sensitization could be expected to lead to relatively low sensitivity, as demonstrated by the quantity of antigen required to elicit responses.

Testing of the crude ASWS fraction on infected guinea pigs (Tables 1 and 2) demonstrated similar specificity and reactivity to that shown on sensitized pigs. However, only 5 μg was required on infected pigs, whereas 50 μg was required on sensitized pigs. The discrepancy in sensitivity could be attributed to either antigen purity or animal sensitivity, or both.

Purification of the components of ASWS antigen led to an antigen that reacted on 100% of our *Blastomyces*-infected test group, with no cross-reaction on the *Histoplasma* test group. The relatively small size (10 to 11 animals/group) may be responsible for these high percentages, but we feel that even in larger groups the figures would not vary significantly. ASWS f_4 produced reactions on the *Blastomyces* group that varied only from 9 to 11 mm in size. The lack of variation of ASWS f_4, while other fractions varied from 0 to 15 mm on the same guinea pigs, adds support to the observed high reactivity rate.

Studies should be undertaken to establish the relation of ASWS f_4 reactivity in infected guinea pigs representing active disease to that in sensitized guinea pigs representing inactive or resolved disease. In addition, the reliability of ASWS f_4 as a skin test antigen for human use should be evaluated. If such investigations lead to positive results, the ASWS f_4 could be an important investigative tool in the detection and diagnosis of blastomycosis.

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